CHAPTER 9

RADIOTHERAPY OF NEOVASCULAR AGE-RELATED MACULAR DEGENERATION; A CLINICAL AND PATHOLOGICAL STUDY

ABSTRACT

Purpose: Radiotherapy has recently been employed to treat patients with neovascular macular degeneration in order to prevent severe visual loss. Radiotherapy affects the evolution of neovascular macular degeneration directly by endothelial toxicity, leading to capillary closure, and/or indirectly through its attenuating effects on the inflammatory response, mediated by macrophages and other inflammatory cells.

Methods*:* In this study we describe the histopathologic findings in a patient with neovascular age-related macular degeneration (AMD) in both eyes whose right eye was treated with radiotherapy (5 times 2 Gy) 3 years before he died. The eyes were enucleated and investigated by light microscopy. Additionally, immunohistochemical investigation with antibodies against CD34 and CD68 was performed to identify patent endothelial cells and macrophages.

Results: Both eyes showed neovascular AMD consisting of mixed fibrocellular and fibrovascular membranes. Capillaries in both the choriocapillaris and the neovascular membrane were patent in both eyes. Macrophages were present in the choroidal neovascularizations of both eyes. Neither preexistent choroidal, intraretinal, nor neovascular vessels showed increased wall thickness as sign of radiation damage.

Conclusion: No radiation-related histopathologic effect could be demonstrated 3 years after radiation therapy in this patient with AMD.

INTRODUCTION

Neovascular age-related macular degeneration (AMD) typically causes a decreased central vision over a short period of time when located subfoveally. This disease is a major cause for visual loss in the elderly population.[3,129] To date the only treatments proven to be successful are subfoveal laser photocoagulation[212] and photodynamic therapy,[213] although both therapies are less effective in patients with occult subfoveal choroidal neovascularization (CNV). Radiotherapy is one of many experimental treatments. Varying results have been published,[314-324] but a recently performed pooled analysis of different studies indicated that radiotherapy may only act to slow or delay the progress of the disease.[216] The mechanism of the effect of ionizing radiation on CNV is not known in detail. Radiotherapy affects the evolution of neovascular macular degeneration directly by endothelial toxicity, leading to capillary closure,[325] and/or indirectly through its attenuating effects on the inflammatory response, mediated by macrophages and other inflammatory cells.[325-327]
Complications of radiotherapy for AMD include radiation retinopathy,[321,328] optic neuropathy,[321,328] cataract[317,318,323] and, recently described, radiation-associated choroidal neovasculopathy after low-dose radiotherapy.[329,330]

This is the first report on histopathological findings after radiotherapy for macular degeneration so far. These findings may help to understand the effect of radiotherapy.

MATERIALS AND METHODS

Case Report
A 67-year-old man was examined in 1990 because of vision loss. Fluorescein angiography showed neovascular AMD in both eyes. Photocoagulation treatment was applied to both eyes, temporally of the fovea. Afterwards, his visual acuity (VA) was 20/40 in his right eye (OD) and 20/20 in his left eye (OS) (Snellen vision), with a low hypermetropic correction. Ophthalmoscopically, the lesions had dried. In May 1991, VA was 20/24 OD and had dropped to 20/240 OS because of a large choroidal neovascular membrane. No additional treatment was instituted. In 1993, patient was referred to our hospital because of a decreased vision in his right eye. His VA was 20/80 OD and 20/200 OS. Fluorescein angiography showed recurrent lesions with early hyperfluorescence at the margins of the old scars. The right eye showed a classical CNV (Figure 9.1A,C), the left eye a classical CNV with occult components (Figure 9.1B,D). Because the patient fixated just at the point of leakage no laser

treatment was given. Instead, he was treated with radiotherapy to his right eye, with a dose of 10 Gy delivered in 5 fractions (Figure 9.2). By 1 month after the treatment patient's VA had dropped further to 20/160 OD. The last clinical examination took place in September 1996; his VA was 20/240 ODS with grade 3 nuclear cataract. On ophthalmoscopy, flat fibrovascular lesions were seen without exudation. In March 1997, the patient died of coronary heart disease at the age of 73 years. Autopsy was permitted and performed within 6 hours. The eyes were removed for histological examination.

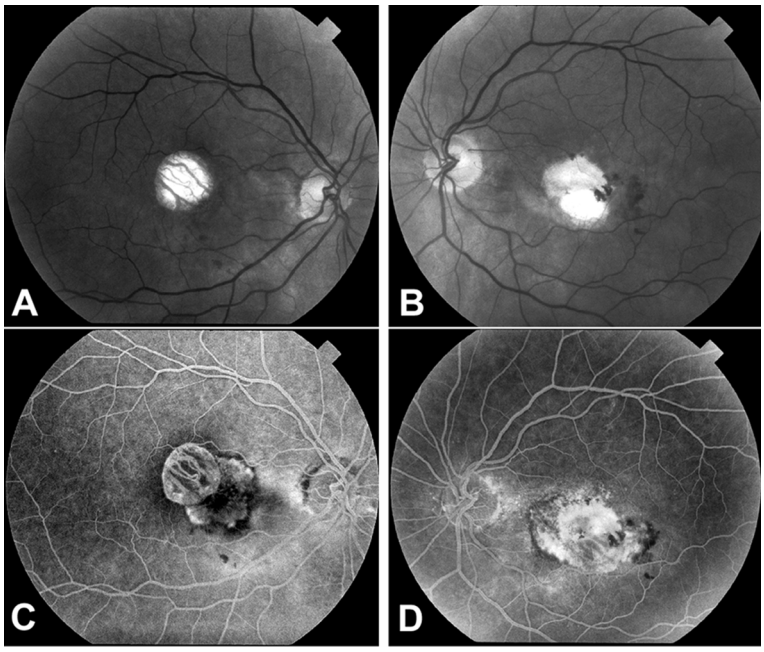

Figure 9.1 Fluorescein angiography 8 months before radiotherapy ***(A,C)*** *Fluorescein angiography of the macular region of the right eye.* ***(A)*** *Red free picture showing areas of atrophy, laser scarring and neovascularization.* ***(C)*** *Early fluorescein angiography of the right eye, showing early hyperfluorescent lesions at the margins of the old scar.* ***(B,D)*** *Fluorescein angiography of the macular region of the left eye.* ***(B)*** *Red free picture showing areas of laser scarring and neovascularization.* ***(D)*** *Early fluorescein angiography, showing a mixed classical and occult lesion with early hyperfluorescence at the margins of the old scar.*

Dosimetry

The patient was treated with radiotherapy to his right eye with a dose of 10 Gy with 16 MeV electrons, in 5 fractions applied with sparing of the lens. The field size was 4 x 4 cm^2 at 100 cm SSD (source skin distance) using a Houston collimating system on a Siemens Mevatron KD-2 linear accelerator. The dose to the macula was calculated using an electron pencil beam model implemented in the Cadplan planning system. The contours are obtained from a CT image. Figure 9.2 shows the calculated isodose pattern. The macula is enclosed by the 100% isodose (2 Gy per fraction).

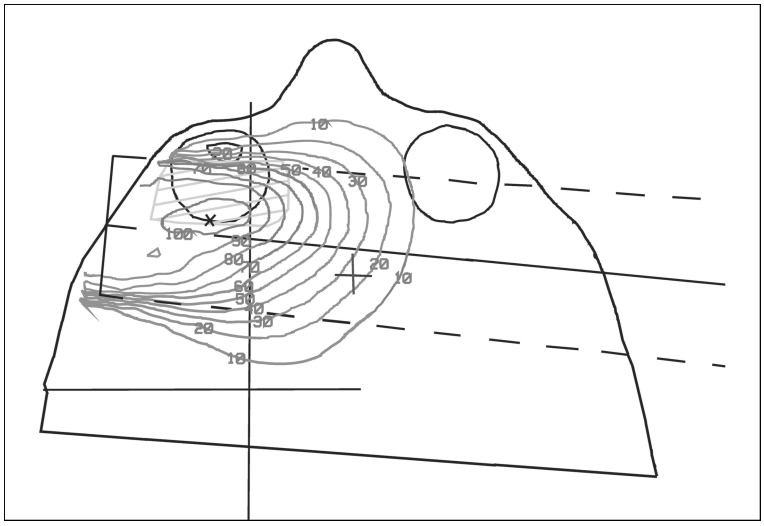

Figure 9.2. *The dose distribution of the 16 MeV electron beam of 4 x 4 cm^2. The macula (x) is enclosed by the 100% isodose (2 Gy per fraction).*

Material preparation and immunohistochemistry

From each enucleated eye, a horizontal tissue block including the macula was excised and horizontally divided in 2 parts. One part was fixed by immersion in formalin for 24 hours and embedded in paraffin. The other part was frozen for other purposes. Five-µm thick paraffin sections were cut and stained for light microscopy with PAS, hematoxylin and eosin, and Mallory. A two-dimensional map was constructed from study of serial sections of both eyes (Figures 9.3A and 9.4A). For immunohistochemistry, monoclonal mouse antibodies against CD34 were obtained from Biogenex (San Ramon, CA, USA) and monoclonal mouse antibodies against CD68 from Dako (Glastrup, Denmark). Immunohistochemical staining was performed as described before.[248] In short, the sections were deparaffinated and rehydrated, and (for CD68) microwave-heated for 10 minutes. After the slides had been blocked with normal goat serum (Dako, 1:10) for 15 minutes, they were incubated with the CD68 antibodies (1:2000) overnight at 4°C, or with CD34 antibodies (1:20) for 1 hour at room temperature. The sections were further incubated with biotinylated multilink antibodies for 30 minutes, followed by alkaline phosphatase-labeled antibiotin (both Biogenex) for 30 minutes. The complex was then visualized by incubating the sections with new fuchsin (as a red chromogen) for 30 minutes in the dark. The slides were counterstained with Mayer's hematoxylin, mounted and examined by light microscopy.

RESULTS

Histopathologic examination

The irradiated right eye (Figure 9.3B to F) showed a subretinal mixed fibrocellular and fibrovascular membrane (Figure 9.3B). The CNV comprised still identifiable RPE and subretinal basal laminar deposits (grade 3[112]). There was extensive loss of photoreceptors at the macular region. Vessels from the choriocapillaris could be demonstrated traversing Bruch's membrane (Figure 9.3D). Immunohistochemistry with CD34, a monoclonal antibody against endothelial cells, showed patent vessels in the choriocapillaris and in the neovascular membrane (Figure 9.3E). Staining with antibodies against macrophages (CD68) showed many macrophages in the CNV and in the underlying choroid (Figure 9.3F). Neither preexistent choroidal, retinal or neovascular vessels showed increased wall thickness as sign of radiation damage. Next to the CNV, a region of laser scarring was seen, with loss of neuroretina, choriocapillaris and choroidal structures (Figure 9.3B).

The left eye (Figure 9.4B to E) showed a large dome-shaped mixed fibrocellular and fibrovascular membrane, with mixed sub-RPE and subretinal areas (Figure 9.4B). Immunohistochemistry with CD34 demonstrated patent vessels in the choriocapillaris and in the neovascular membrane (Figure 9.4E). Staining with antibodies against CD68 showed many macrophages in the CNV and in the underlying choroid (not shown). The overlying neuroretinal layers were disorganized and atrophic. The CNV was partly overlying a region of laser scarring, with loss of choriocapillaris and choroidal structures (not shown).

DISCUSSION

In this patient with bilateral neovascular AMD we showed that, 3 years after 10 Gy radiotherapy to the posterior pole of his right eye, choriocapillaries and neovascular capillaries were still patent and macrophages were present. The dose given is at the low end of the range of presently applied protocols.[216] There were no histologic signs of radiation effect on the preexistent vascular walls. This may be due to the relatively low dose of radiation or to the reversibility of minor damage.

Radiotherapy affects the evolution of neovascular macular degeneration by endothelial toxicity, resulting in narrowing or occlusion of blood vessels.[325-327] Endothelial cells are moderately sensitive to radiation and vessels may reveal manifestations of radiation injury months to years later.[325,331-333] In CNV, microvessel and endothelial cell loss

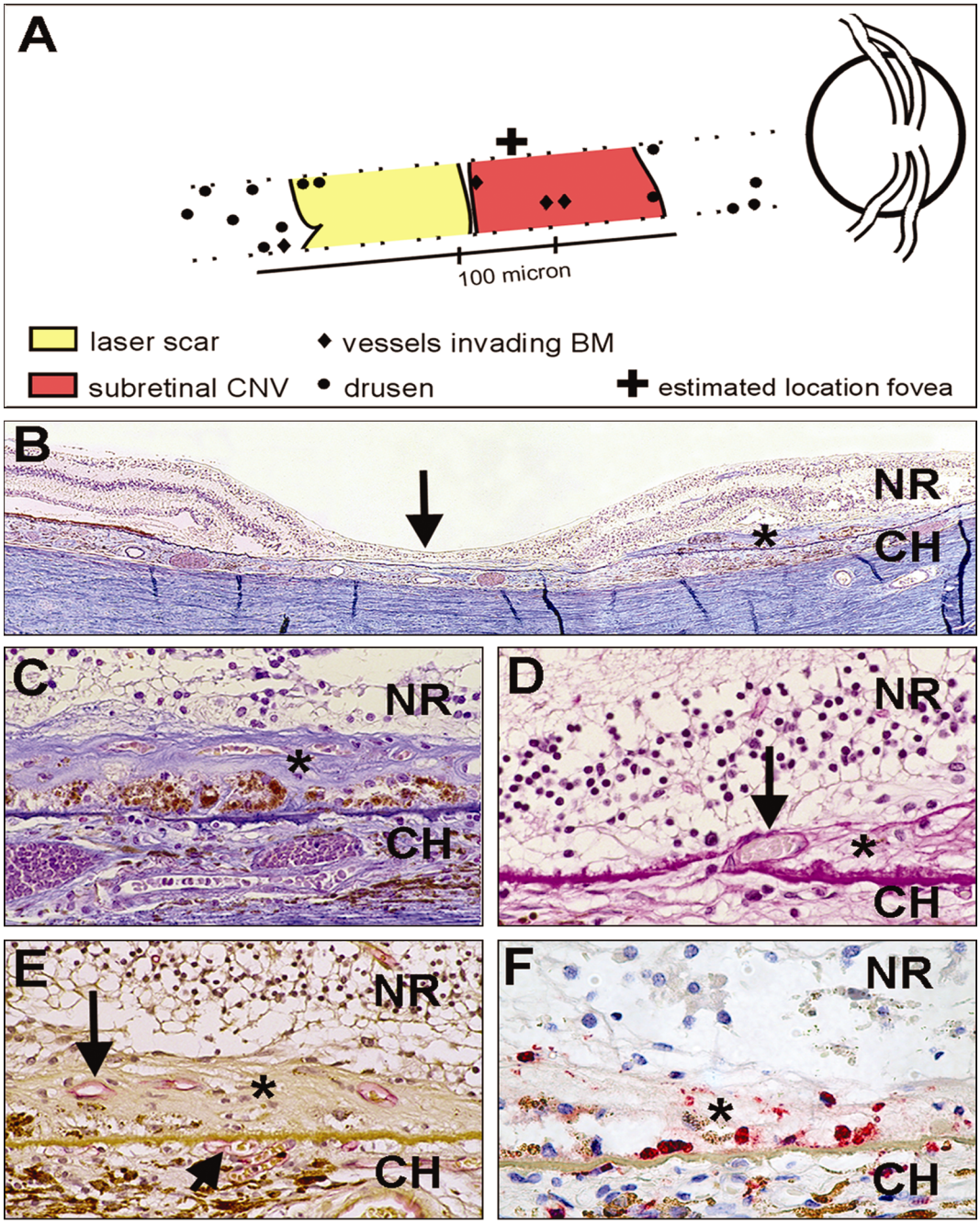

Figure 9.3 Irradiated right eye. (A) *Two-dimensional reconstruction map showing size, shape and location of selected histopathologic features of the examined part of the macular region.* ***(B)*** *Histologic composition of macular region, showing a CNV membrane (*). A region of laser scarring is seen (arrow), with total atrophy of neuroretina, choriocapillaris and disturbance of choroidal structures (Mallory, original magnification x100).* ***(C)*** *Detail of CNV showing subretinal, fibrovascular region with intact choriocapillaris. (Mallory, original magnification x400).* ***(D)*** *At the margin of the CNV a vessel from the choriocapillaris traverses Bruch's membrane (arrow) (PAS, original magnification x400).* ***(E)*** *Immunohistochemical staining with antibodies against CD34, a marker for endothelial cells, with a red chromogen. Patent choriocapillaries (short arrow), as well as patent neovascular capillaries (long arrow) in the CNV are seen (original magnification x400).* ***(F)*** *Immunohistochemical staining with antibodies against CD68, a marker for macrophages, with a red chromogen shows many macrophages in the CNV as well as in the choroid (original magnification x400). * = choroidal neovascularization; NR = neuroretina; CH = choroid.*

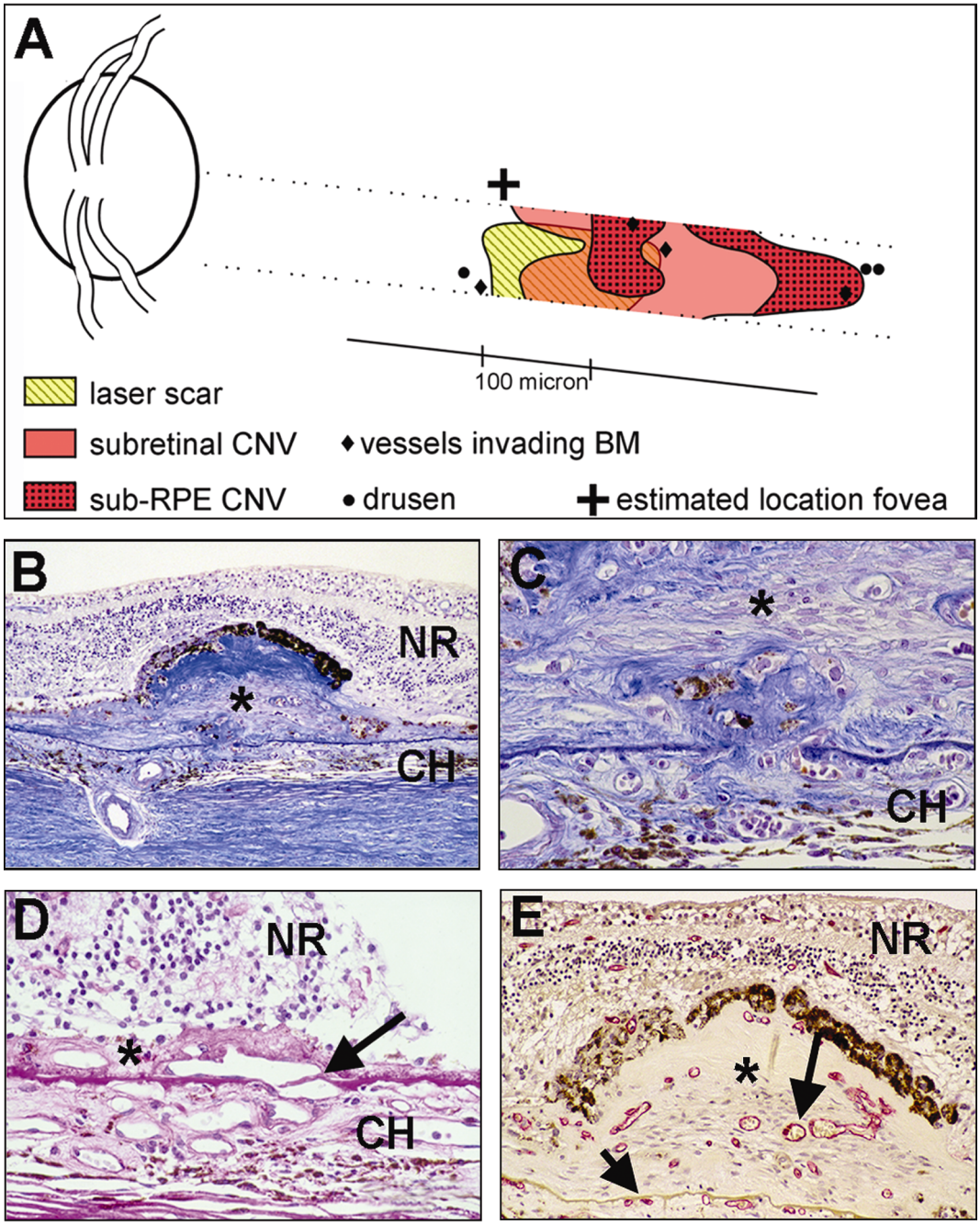

Figure 9.4 Fellow left eye. ***(A)*** *Two-dimensional reconstruction map showing size, shape and location of selected histopathologic features of the examined part of the macular region.* ***(B)*** *Histologic overview of a dome-shaped CNV membrane with a large sub-RPE region. The overlying retina is disorganized. (Mallory, original magnification x200).* ***(C)*** *Detail of CNV showing intact choriocapillaris and vessels traversing Bruch's membrane (Mallory, original magnification x400).* ***(D)*** *At the margin of the CNV a vessel from the choriocapillaris traverses Bruch's membrane (PAS, original magnification x400).* ***(E)*** *Immunohistochemical staining with antibodies against CD34. Patent choriocapillaries (short arrow), as well as patent neovascular capillaries (long arrow) in the CNV are seen (original magnification x400). * = choroidal neovascularization; NR = neuroretina; CH = choroid.*

occurs about a year after irradiation.[325] Moreover, CNV membrane regression is not found until 6 months or more after radiotherapy, independent of the dose administered.[315] Since radiotherapy in our patient was performed three years before

histologic examination, it appears valuable to assess possible vascular damage as morphologic parameter for radiation damage.

A more rapid effect of ionizing radiation on neovascular macular degeneration is to be expected through its attenuating effects on the inflammatory response, mediated by macrophages and other inflammatory cells.[326,327] In our patient macrophages were similarly present in both eyes in the CNV as well as in the underlying choroid, three years after irradiation of the right eye. Therefore, it appears unlikely that the presence of these macrophages can be attributed to an immediate effect of the irradiation.

The histopathologic effect of radiotherapy has not been documented in cases of human macular degeneration so far. Miyamoto et al.[334] studied the histologic appearance of rabbit eyes with experimental CNV 4 weeks after a single fraction of 20 Gy of focal X-irradiation. The degree of vascular formation and the number of vascular endothelial cells in the subretinal membrane of the irradiated eyes were less than in those of control eyes. However, the pathogenesis of experimental CNV in rabbit eyes may not be identical to that of CNV in AMD. Furthermore, a single fraction of 20 Gy has different effects on choroidal endothelial cells than a fractionated dose.[335]

Clinical trials on radiotherapy show a probable benefit with higher doses.[314,317,318] Other clinical studies demonstrate similar results between treated and controls after lower-dose radiotherapy and longer follow-up.[319,322,324] In a pooled analysis of data from independent centers, fraction size was not found responsible for variation in visual outcome.[216] Our results are in concordance with the findings of little effect of low dose radiotherapy on CNV at longer follow-up.

With low-dose radiotherapy, few side effects are to be expected. However, recently a vasculopathy has been described, developing within months after low-dose radiotherapy (10 to 20 Gy),[329,330] called radiation-associated choroidal neovasculopathy.[330] The affected patients appeared to have a particularly poor visual prognosis. Our patient does not appear to belong to the (still poorly described) subset of patients who develop the vasculopathy.

The histologic significance of the findings on fluorescein angiography must be interpreted with care, because fluorescein angiography was not performed after radiotherapy. However, the clinical findings were well documented at regular intervals between fluorescein angiography and the last ophthalmoscopy and no obvious changes were recorded. At the last ophthalmoscopy, a flat fibrovascular lesion was seen without exudation, which is in accordance with the histologic findings.

In conclusion, no radiation-related histopathologic effect could be demonstrated 3 years after radiotherapy (10 Gy) in this patient with neovascular AMD.

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